PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Stevens, Fred J. et al.

Application: "FIBRIL-BLOCKING PEPTIDE, A METHOD FOR PREVENTING FIBRIL FORMATION"

Serial No.: 09/712,819

Filed: November 13, 2000

Art Unit: 1644

Examiner: Dr. Phuong N. Huynh, Ph. D.

CERTIFICATE OF MAILING: I hereby certify that this correspondence is being deposited with the United States Postal Service pursuant to 37 C.F.R. 1.8 as first class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 on **January 29, 2003** (Date of Deposit)

Michael J. Cherskov — Name of Representative

Signature of Representative — 1-29-03 Date of Signature

Assistant Commissioner for Patents
Washington, D.C. 20231

20 North Wacker Drive
Chicago, IL 60606
312-621-1330

37 CFR 1.131 Affidavit of Fred J. Stevens

Dear Sir:

Dr. Fred J. Stevens, being first duly sworn, deposes and says that:

1. I am a joint inventor of the invention described and claimed in the above-identified patent application.

2. I declare that conception of the invention occurred in the United States.

3. I understand that the Patent Examiner is using an October 1999 article by Davids *et al*, published in the Journal of Immunology to reject the anti-aggregation methods and peptide claimed in my patent application. However, I declare that I possessed the knowledge and otherwise reduced to practice the claimed invention before October 1999. To with:

4. My method for minimizing the aggregation tendencies of an amyloid forming protein, a method for preventing amyloid formation in human kappa-IV light chain variable domain, and a peptide for insertion in an intact human kappa-IV light chain variable domain as described and claimed in the instant application was reduced to practice at least as early as January 12, 1999. An invention report (copy attached hereto as Exhibit A) describing an embodiment of the invention was prepared by myself and other scientists on January 12, 1999 at Argonne National Laboratory. An invention disclosure record was signed by myself and a co-inventor on April 5, 1999 (copy attached hereto as Exhibit B).

5. My method for minimizing or preventing the aggregation or fibril assembly of an amyloid forming protein such as human immunoglobulin light chain variable domain and a peptide with an ability to block fibril formation by antibody light chain variable domains were known and reduced to practice at least as early as January 12, 1999, as evident by their disclosure within the invention report.

6. More specifically, the following information regarding this invention was known and reduced to practice as early as January 12, 1999:
The invention report teaches a peptide Phe_{71}-Thr_{72}-Leu_{73}-Thr_{74}-Ile_{75}-Ser_{76}-Ser_{77}-Leu_{78} of the human kappa-IV light chain variable domain (page 1). The peptide demonstrates the ability to block fibril formation (page 1).

7. Also, the invention report teaches that fibril assembly is interrupted by addition of the small peptide (page 1). The peptide inserts itself into a region of the light chain variable domain structure that is exposed following partial unfolding of the domain. By occupying and inserting itself into the complementary region, the peptide prevents a domain swapping event. The peptide is identical in composition to a portion of the light chain variable domain that is critical for stable packing of the molecule.

8. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and, further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and may jeopardize the validity of the aforesaid patent application.

Date: 01/24/03

Fred J. Stevens

ARGONNE NATIONAL LABORATORY
9700 South Cass Avenue, Argonne, Illinois 60439

April 7, 1999

Mr. Robert J. Fisher
Deputy Chief Counsel
Office of Intellectual Property Law
U.S. Department of Energy
9800 South Cass Avenue
Argonne, Illinois 60439

Re: **ANL Case #:** ANL-IN-99-019
Inventors: Fred J. Stevens, Yair Argon, David Davis, and Rosemarie Raffen
Title: **A FIBRIL-BLOCKING PEPTIDE**

Dear Mr. Fisher:

Enclosed for your records is a signed Invention Report for the captioned case. Please keep us advised of the status of this invention and let us know the DOE file number assigned to it.

Sincerely,

Sandra A. Classen

Sandra A. Classen
Legal Department

SAC:cs
Encls.

ct: Brian Frost
Steve Lake
Carol Giometti

INVENTION REPORT

ANL CASE #: ANL-IN-99-019

DOE CASE #:

INVENTORS: Fred J. Stevens
Yair Argon
David Davis
Rosemarie Raffen

TITLE: **A FIBRIL-BLOCKING PEPTIDE**

DESCRIPTION: This invention discloses the composition of an eight amino acid peptide that demonstrates the ability to block the variable domains, *in vitro* fibril formation by antibody light chain variable domains.

In the class of diseases, called "conformational disease", several conditions are thought to share a common etiology in the improper folding or aggregation of proteins that results in one or more structural or conformational flaws. These resulting conformational flaws often contribute to the onset of diverse disease conditions such as sickle cell anemia, amyloid light chain disease, senile systemic amyloidosis, Alzheimer's disease, and the transmissible prion encephalopathies, including kuru and "mad cow" disease or BSE.

No drug has been developed to treat these conformational disease phenomena at the level of intervention in the protein aggregation process. This invention describes an eight amino acid peptide (Phe-Thr-Leu-Thr-Ile-Ser-Leu), for which properties antagonistic to antibody light chain amyloid fibril formation have ben demonstrated. Peptides of other sequence have been tested and are not effective antagonists. Although it is probable that alternative compositions could block fibril formation, the lack of activity by other peptides tested indicates a necessary structural specificity.

The mechanism by which this peptide inhibits the amyloid fibril formation is speculated to involve a "domain swapping event". The insertion of the peptide into a particular groove occupied by an adjacent fibril subunit is thought to interrupt fibril assembly and thus prevent fibril formation.

Further experiments are underway to test this hypothesis that could result in the identification of a new class of therapeutic compounds and the elucidation of a method by which such compounds could be screened for efficacy as antagonists of fibril formation.

The utility of this particular composition to prevent light chain fibril formation, *in vitro*, has been demonstrated in the laboratory.

BACKGROUND, INCLUDING RELATED ART: See report by Stevens *et al.*, and abstracts by Carrell and Gooptu and Peterson *et al.*

PUBLICATIONS, REPORTS AND TALKS: None planned at this time.

INVENTORS' STATUS: The inventors, Fred J. Stevens, and Rosemarie Raffen, are employed by Argonne National Laboratory in the Center for Mechanistic and Biotechnology Division. Yair Argon and David Davies are employed by The University of Chicago. They are citizens of the United States. This invention was conceived under Contract No. W-31-109-ENG-38 between the U.S. Department of Energy (DOE) and The University of Chicago representing Argonne National Laboratory.

BADGE NUMBERS: 27475 Fred Stevens; 45318 Rosemarie Raffen

FUNDING SOURCE for research under which invention was conceived:

ANL: ___ LDRD (Laboratory Director Research & Development, previously called Exploratory Research Funds [ERF] or Program Development Funds [PDF])

Were LDRD (or ERF/PDF) funds used to support research that preceded the research during which the invention was conceived? ___ No _X_ Yes

Cost code under which invention was conceived: 85742-00-110 61000-20-110 B&R Code: 60-03 KP-11-01

Non-ANL/DOE Sponsor: Name of organization: NIH

Type of organization: _X_ Federal ___ State ___ Private ___ Not-for-profit

Type of funding document or agreement: _X_ WFO ___ CRADA ___ HTSCA ___ MIPR

Other (specify): _____ Agreement No.: _____

PROBABLE VALUE: Possible therapeutic agent for amyloid fibril diseases.

RECOMMENDATIONS: The recommendations of ITD personnel will be provided later.

EXCEPTIONAL CIRCUMSTANCES: This invention is not an exceptional circumstance invention.

REPORT DATED: February 24, 1999 - Victoria Henson-Apollonio

READ AND UNDERSTOOD BY:

[signature]	4/5/99	[signature]	4.5.99
Inventor Signature	Date	Witness Signature	Date
[signature]	4-5-99	[signature]	4.5.99
Inventor Signature	Date	Witness Signature	Date
Inventor Signature	Date	Witness Signature	Date
Inventor Signature	Date	Witness Signature	Date

Composition of a fibril-blocking peptide; strategy for development of peptides to inhibit conformational disease resulting from protein domain swapping.

Fred J. Stevens[a]
Yair Argon[b]
David Davies[b]
Rosemarie Raffen[a]

[a]**Center for Mechanistic Biology and Biotechnology, Argonne National Laboratory**

[b]**University of Chicago**

01/12/99

The term "conformational diseases" refers to a class of pathologies that originates from improper folding and/or aggregation of proteins that usually exhibit one or more structural (or conformational) flaws. Such diseases include sickle cell anemia in which a single amino acid variation renders hemoglobin prone to aggregation when deoxygenated. This leads to deformation of the red blood cell and impaired circulation through the capillaries. Other conformational diseases are less familiar. In the amyloidoses such as those resulting from antibody light chains (amyloid AL) and transthyretin (familial amyloidosis, senile cardiac amyloidosis) proteins form highly ordered fibrillar assemblies that accumulate in tissues and organs and result in death. Amyloids are also found in Alzheimer's disease and the prion-related spongiform encephylactic diseases (kuru, "mad cow" disease) but the role of the fibrils remains unresolved. The most widely known conformational disease is that of cataracts involving age-related aggregation of molecules of the crystalline family of proteins in the lens of the eye.

No drug has been developed to treat these conformational disease phenomena at the level of intervention in the protein aggregation process. We have now identified an eight amino acid peptide that demonstrates the ability to block fibril formation (by antibody light chain variable domains) in vitro. Although work remains to validate the generality of the observation and it is premature to argue that this peptide represents a therapeutically useful drug, this finding is significant in that it demonstrates the feasibility of interrupting fibril assembly by addition of a small molecule. First, this peptide could, in fact, possibly work as a therapeutically useful drug or be could be modified or mimicked to generate a compound that would work as an effective antagonist of fibril formation. Second, this peptide will be of substantial value as a research tool with which to obtain further insight into the mechanism of fibril formation. Third, a rational mechanism for the activity of the peptide can be formulated in which the peptide inserts itself into a region of the light chain variable domain structure that is exposed following partial unfolding of the domain. By occupying and inserting itself into the complementary region, it is possible that a "domain swapping event" in which the corresponding portion of a second variable domain inserts itself is prevented. This type of domain swap has been observed in other proteins and would link together two variable domains into a hybrid unit that could be an intermediate in fibril development. Fourth, because the peptide is identical in composition to a portion of the light chain variable domain that is critical for stable packing of the molecule, the observation suggests that it may be possible to generalize this strategy of structural mimicry to develop other peptides that may serve as antagonists (inhibitors) of other conformational disease processes.

The peptide that we have identified as inhibitory of fibril formation in vitro comprises positions 71 through 78 of the human kappa-4 light chain variable domain. Peptides of other sequence have been tested are not effective. Although it is probable that alternative compositions could antagonize fibril formation, the lack of activity by other peptides tested indicates structural specificity. The functional sequence is

Phe_{71}-Thr_{72}-Leu_{73}-Thr_{74}-Ile_{75}-Ser_{76}-Ser_{77}-Leu_{78}

The numbers indicate the positions of these residues in an intact human kappa-IV light chian variable domain. Positions Leu73, Ile75, and Leu78 may be key; many substitutions at other sites are likely to be compatible with a functional peptide. These highly conserved side chains are inserted into the core of the variable domain and provide the major anchorage for a hairpin turn spanning residues from position 60 through 83. It has been well established that mutations that compromise a highly conserved ionic interaction between residues at positions 61 and 82 are highly correlated with amyloidosis, possibly by compromising the interaction between the hairpin and the domain core. If this interaction is compromised, one envisions that the hairpin turn can more readily diffuse away from the core of the domain. This would provide an opportunity for the extruded portion of the protein (component A) to insert itself into the corresponding portion of a second molecule (component B). Likewise, the cavity formed in A could be filled by the extruded portion of component B, resulting in an AB dimer in which the polypeptide chains are intermingled. It would be expected that a fibrillar assembly in which the exchanged portions of the molecules are buried would be highly stable.

The mechanism outlined above might also apply to other amyloidogenic proteins that share with variable domains a structural motif known as a greek key. This motif is characterized by a so-called beta sheet sandwich in which one or more of the "hairpin" turn structures are found. Amyloid forming proteins such as antibody constant domains, transthyretin, and beta-2-microglobulin are all of this motif and it may be appropriate to collectively describe this class of amyloid forming proteins as constituting the "greek key amyloidoses." Serpins (serine protease inhibitors) while not formally greek key proteins, are amyloid forming proteins known to be capable of domain exchange. It is possible that peptides that mimic appropriate portions of the internal structure of any of these proteins might be effective in inhibiting fibril formation. Although the composition of therapeutic peptides are likely to be disease-specific, the approach to developing the therapeutic agents should be common to all greek key amyloidoses.

Cataracts are formed by inappropriate aggregation of "crystalline" proteins in the lens of the eye. Although it is not suggested that this is an amyloid-like process, it is of interest that crystallines are also greek key proteins. These proteins are "long-lived" and accumulate damage during the life-time of the individual. It is readily imaginable that oxidative and other reactions involving the protein can be considered the chemical equivalent to genetic mutation, leading to destabilization and increased tendency to undergo partial unfolding and, with it, the attendant risk of domain swaps that may serve to nucleate the formation of light scattering aggregates in the eye. It is possible that the strategy suggested herein for the development of molecules to control greek key amyloidosis may also be relevant to other diseases involving inappropriate protein-protein relationships.

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Curr Opin Struct Biol 1998 Dec;8(6):799-809

Conformational changes and disease--serpins, prions and Alzheimer's.

Carrell RW, Gooptu B

Department of Haematology Cambridge Institute for Medical Research University of Cambridge Hills Road Cambridge CB2 2XY UK. rwc 1000@cam.ac.uk

Some of the most perplexing disorders in medicine are each now known to arise from the conformational instability of an underlying protein. The consequence is a continuum of pathologies with typically a change in fold leading to ordered aggregation and tissue deposition. The serpins provide a structural prototype for these pathologies and give a perspective on the assessment of current proposals as to the conformational basis of both Alzheimer's disease and the transmissible prion encephalopathies.

Publication Types:

- Review
- Review, tutorial

PMID: 9914261, UI: 99116082

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Proc Natl Acad Sci U S A 1998 Oct 27;95(22):12956-60

Inhibiting transthyretin conformational changes that lead to amyloid fibril formation.

Peterson SA, Klabunde T, Lashuel HA, Purkey H, Sacchettini JC, Kelly JW

Department of Chemistry and Skaggs Institute of Chemical Biology, Scripps Research Institute, 10550 North Torrey Pines Road MB 12, La Jolla, CA 92037, USA.

Insoluble protein fibrils resulting from the self-assembly of a conformational intermediate are implicated as the causative agent in several severe human amyloid diseases, including Alzheimer's disease, familial amyloid polyneuropathy, and senile systemic amyloidosis. The latter two diseases are associated with transthyretin (TTR) amyloid fibrils, which appear to form in the acidic partial denaturing environment of the lysosome. Here we demonstrate that flufenamic acid (Flu) inhibits the conformational changes of TTR associated with amyloid fibril formation. The crystal structure of TTR complexed with Flu demonstrates that Flu mediates intersubunit hydrophobic interactions and intersubunit hydrogen bonds that stabilize the normal tetrameric fold of TTR. A small-molecule inhibitor that stabilizes the normal conformation of a protein is desirable as a possible approach to treat amyloid diseases. Molecules such as Flu also provide the means to rigorously test the amyloid hypothesis, i.e., the apparent causative role of amyloid fibrils in amyloid disease.

PMID: 9789022, UI: 99007248

INVENTION DISCLOSURE RECORD AND EVALUATION
ANL Case N . ANL-IN-99-019

The invention report appended hereto will serve to describe formally the invention.

1. Title of Invention: **A FIBRIL-BLOCKING PEPTIDE**

2. Brief Description of Invention Emphasizing Unique and Novel Aspects (attach separate sheet if necessary):

The specific invention is the composition of matter of a linear peptide Phe-Thr-Leu-Thr-Ile-Ser-Ser-Leu that has demonstrated the capability to block in vitro fibril formation by antibody light chain variable domains. This peptide corresponds to a fragment of the intact protein.

The broader concept involves: (a) use of the peptide itself (or analog) as a drug for treatment of antibody light chain amyloidoses; (b) use of peptides that mimic other portions of the molecule to identify other possible sites ~~for~~ ~~this~~ as targets for drug design; (c) extension of this strategy to several other proteins that form amyloid fibrils in patients and are structurally similar to the antibody light chain in that they share a "Greek key" folding motif. Note, also, that recent modeling study (Chaney et al., Protein Eng. 11: 761-767) has suggested that the A-beta peptide associated with fibril formation in Alzheimer's disease also has a Greek key" folding motif.

3. Dates and Places of Inventions: 1/12/99 ANL			
Conception by Inventor		At: ANL	
First Sketch or Drawing:	At:	In Workbook: 1	Page:
First Written Description:	At:	In Workbook:	Page:
Disclosure to Others: report of 1/12/99			
		At:	
		At:	
		At:	
Completion of Model or Full Size Device:		At:	

4.. Has your invention been reduced to practice? Y/N. If yes, what was the performance of the invention?

Y: — in terms of in vitro testing

If no, what further development work is needed to reduce the invention to practice and who will fund it? (Include an estimate of the cost and manpower that will be needed.)

N: Further work is needed to establish pharmaceutical potential resulting from this lead compound — and to extend the approach to other forms of amyloidosis

5. What are the potential uses of this invention by the government and/or by industry? What needs does it fulfill? What advantages does it have over existing products or processes?

The invention itself may be used as a basic research tool for study of amyloid disease and as a starting point for drug development.

6. List any industry contacts who have shown an interest in this invention or this line of work. Have you discussed the possibility of collaborative development through a CRADA? Did you use a non-disclosure agreement? – Maybe

MediChem Research Inc (Lemont IL)

7. Have you disclosed this invention and/or what plans do you have to disclose aspects of this invention - publications, talks, public use? Please give the dates.

Publication to be submitted within 2 months

8. List other R&D organizations working in the technology area of your invention, including key names where possible.

Numerous academic and commercial entities researching amyloidosis and related disease. Primary focus is Alzheimer's disease.

9. Do you recommend foreign filing of this invention? If yes, what countries?

Yes, Developed countries.

10. Inventor (PLEASE PRINT OR TYPE):

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Work Phone #	: 773-702-9185
ANL Badge #	:
Div./Bldg	:

11. Signature of Inventor and Date:	Signature of Witness and Date:

(Witnesses are attesting they understand the description of the invention and that the signatures of the inventors are valid.)